

20	76	5.8	440	7	US-11-796-730-3929	Sequence 3929, Ap
21	76	5.8	815	7	US-11-551-744-236	Sequence 236, App
22	75	5.8	269	7	US-11-796-730-2989	Sequence 2989, Ap
23	75	5.8	514	7	US-11-336-426-34	Sequence 34, Appl
24	74.5	5.7	616	6	US-10-574-194-39	Sequence 39, Appl
25	74.5	5.7	617	6	US-10-574-194-41	Sequence 41, Appl
26	74	5.7	828	7	US-11-417-216-10	Sequence 10, Appl
27	73.5	5.6	467	7	US-11-649-663A-3270	Sequence 3270, Ap
28	73	5.6	416	7	US-11-796-730-2132	Sequence 2732, Ap
29	72.5	5.6	356	6	US-10-438-246-8715	Sequence 8715, Ap
30	72.5	5.6	463	7	US-11-796-730-5223	Sequence 5223, Ap
31	72	5.5	234	7	US-11-796-730-2847	Sequence 2847, Ap
32	71.5	5.5	218	7	US-11-698-831-23	Sequence 23, Appl
33	71.5	5.5	238	7	US-11-796-730-3381	Sequence 3381, Ap
34	71.5	5.5	245	7	US-11-796-730-3960	Sequence 3580, Ap
35	71.5	5.5	489	7	US-11-796-730-2808	Sequence 2806, Ap
36	71.5	5.5	544	7	US-11-796-730-3538	Sequence 3538, Ap
37	71.5	5.5	1621	6	US-10-438-246-15843	Sequence 15843, A
38	71	5.5	389	7	US-11-788-342-54	Sequence 54, Appl
39	70.5	5.4	483	7	US-11-649-663A-5500	Sequence 5500, Ap
40	70	5.4	267	6	US-10-533-069-1272	Sequence 1272, Ap
41	70	5.4	824	7	US-11-551-744-233	Sequence 233, App
42	69.5	5.3	299	7	US-11-649-663A-2878	Sequence 2878, Ap
43	69.5	5.3	331	7	US-11-726-100-3	Sequence 3, Appli
44	69.5	5.3	436	7	US-11-796-730-4125	Sequence 4125, Ap
45	69.5	5.3	468	6	US-10-438-246-32290	Sequence 32290, A

Attachment

Case# 10 / 724,264

## ALIGNMENTS

## RESULT 1

US-10-438-246-18926  
; Sequence 18926, Application US/10438246  
; Publication No. US20070192889A1  
; GENERAL INFORMATION:  
; APPLICANT: La Rosa, Thomas J.  
; APPLICANT: Lutfiyya, Linda L.  
; APPLICANT: Zhou, Yihua  
; APPLICANT: Kovalic, David K.  
; APPLICANT: Barbazuk, Brad  
; APPLICANT: Li, Ping  
; APPLICANT: Wu, Wei  
; APPLICANT: Boukharov, Andrey A.  
; TITLE OF INVENTION: Nucleic Acid Molecules and Other Molecules Associated with  
; TITLE OF INVENTION: Transcription in Plants and Uses Thereof for Plant Improvement  
; FILE REFERENCE: 38-21(5333)B  
; CURRENT APPLICATION NUMBER: US/10/438,246  
; CURRENT FILING DATE: 2003-05-14  
; NUMBER OF SEQ ID NOS: 33516  
; SEQ ID NO 18926  
; LENGTH: 1691  
; TYPE: PRT  
; ORGANISM: Oryza sativa  
; FEATURE:  
; OTHER INFORMATION: Clone ID: TF\_OSC416395j.C1.p15.fg  
US-10-438-246-18926

Query Match 7.6%; Score 98.5; DB 6; Length 1691;  
Best Local Similarity 22.4%; Pred. No. 0.022;  
Matches 58; Conservative 41; Mismatches 91; Indels 69; Gaps 13;

Qy	3 LLLILGSVIALPTF-----AAGGGDLDASDYTG-VSFVLVTAALLASTVFFFVERDRV 54
:   :     :     :     :   :   :   :   :   :   :   :   :   :   :	
Db	126 LVLFCACAVILLPVYIFTTPLLIALGQDPEISAVAGTISLWYI--PVMFSYIWAFMLQMYL 183
Qy	55 SAKWKTSLTSGLVTFGIAFWHYMMRGVWIETGDSPTVFRYIDWLTVPLICEFYLILA 114
:   :   :   :   :   :   :   :   :   :   :   :   :   :   :   :   :	
Db	184 QAQSKNM-----IVTYLAF-----LNLG----KHLFLSWLLTV---KFQLGLA 219
Qy	115 AATNVAGSLFKKLGVGSLLVMLVFGYMGEAGIMAAWPAPIIGCLAWVYMIYELWAGEGKSA 174
:   :   :   :   :   :   :   :   :   :   :   :   :   :   :   :	
Db	220 ---GVMGSMVISFWIPVFCQLAFVFFG--GCPLWTGFSSAFTDLGAIMKLSLSSGVML 274
Qy	175 CNTASPAVQSAYNNTMMYIIIFGWAIYPVGYFTGYLMGD--GGSALNLNLIY----- 223
:   :   :   :   :   :   :   :   :   :   :   :   :   :   :   :	
Db	275 C-----LELWYNTILVLL-----TGYMKNAEVALDALSICLAYIFTESKAVA 316
Qy	224 -NLADFVNKILFGLIWNV 241
:  :   :   :	
Db	317 DEVADLAPLLAFASSILLNSV 335

## RESULT 2

US-10-438-246-25027  
; Sequence 25027, Application US/10438246  
; Publication No. US20070192889A1  
; GENERAL INFORMATION:  
; APPLICANT: La Rosa, Thomas J.  
; APPLICANT: Lutfiyya, Linda L.  
; APPLICANT: Zhou, Yihua

10/784,264

=> d his

(FILE 'HOME' ENTERED AT 12:26:36 ON 18 SEP 2007)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,  
LIFESCI' ENTERED AT 12:27:04 ON 18 SEP 2007

L1        318 S PROTEORHODOPSIN  
L2        86 S "HISTIDINE 75" OR "HIS75"  
L3        0 S L1 AND L2  
L4        0 S "H75" AND L1  
            E JENSEN R B/AU  
L5        150 S E3  
            E KELEMEN B/AU  
L6        102 S E1-E12  
L7        10 S L1 AND L6  
L8        5 DUP REM L7 (5 DUPLICATES REMOVED)  
L9        6 S L1 (W) MUTANT?  
L10      2 DUP REM L9 (4 DUPLICATES REMOVED)

=

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PASSWORD :

TERMINAL (ENTER 1, 2, 3, OR ?):2

\* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* Welcome to STN International \* \* \* \* \* \* \* \* \* \* \* \* \* \* \*

NEWS 1 Web Page for STN Seminar Schedule - N. America  
NEWS 2 JUL 02 LMEDLINE coverage updated  
NEWS 3 JUL 02 SCISEARCH enhanced with complete author names  
NEWS 4 JUL 02 CHEMCATS accession numbers revised  
NEWS 5 JUL 02 CA/CAplus enhanced with utility model patents from China  
NEWS 6 JUL 16 CAplus enhanced with French and German abstracts  
NEWS 7 JUL 18 CA/CAplus patent coverage enhanced  
NEWS 8 JUL 26 USPATFULL/USPAT2 enhanced with IPC reclassification  
NEWS 9 JUL 30 USGENE now available on STN  
NEWS 10 AUG 06 CAS REGISTRY enhanced with new experimental property tags  
NEWS 11 AUG 06 BEILSTEIN updated with new compounds  
NEWS 12 AUG 06 FSTA enhanced with new thesaurus edition  
NEWS 13 AUG 13 CA/CAplus enhanced with additional kind codes for granted patents  
NEWS 14 AUG 20 CA/CAplus enhanced with CAS indexing in pre-1907 records  
NEWS 15 AUG 27 Full-text patent databases enhanced with predefined patent family display formats from INPADOCDB  
NEWS 16 AUG 27 USPATOLD now available on STN  
NEWS 17 AUG 28 CAS REGISTRY enhanced with additional experimental spectral property data  
NEWS 18 SEP 07 STN AnaVist, Version 2.0, now available with Derwent World Patents Index  
NEWS 19 SEP 13 FORIS renamed to SOFIS  
NEWS 20 SEP 13 INPADOCDB enhanced with monthly SDI frequency  
NEWS 21 SEP 17 CA/CAplus enhanced with printed CA page images from 1967-1998  
NEWS 22 SEP 17 CAplus coverage extended to include traditional medicine patents  
  
NEWS EXPRESS 05 SEPTEMBER 2007: CURRENT WINDOWS VERSION IS V8.2, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 05 SEPTEMBER 2007.  
  
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FILE 'HOME' ENTERED AT 12:26:36 ON 18 SEP 2007

=> file medline embase biosis biotechds scisearch hcaplus ntis lifesci  
COST IN U.S. DOLLARS

	SINCE FILE	TOTAL
	ENTRY	SESSION
FULL ESTIMATED COST	0.21	0.21

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FILE 'LIFESCI' ENTERED AT 12:27:04 ON 18 SEP 2007  
COPYRIGHT (C) 2007 Cambridge Scientific Abstracts (CSA)

=> s proteorhodopsin  
L1 318 PROTEORHODOPSIN

=> s "histidine 75" or "his75"  
L2 86 "HISTIDINE 75" OR "HIS75"

=> s l1 and l2  
L3 0 L1 AND L2

=> s "H75" and l1  
L4 0 "H75" AND L1

=> e jensen r b/au  
E1 21 JENSEN R A C/AU  
E2 1 JENSEN R A JR/AU  
E3 150 --> JENSEN R B/AU  
E4 5 JENSEN R B B/AU  
E5 1 JENSEN R BUGGE/AU  
E6 111 JENSEN R C/AU  
E7 68 JENSEN R D/AU  
E8 2 JENSEN R DUANE/AU  
E9 387 JENSEN R E/AU  
E10 1 JENSEN R E \*/AU  
E11 1 JENSEN R E V/AU  
E12 2 JENSEN R F/AU

=> s e3  
L5 150 "JENSEN R B"/AU

```

=> e kelemen b/au
E1          9      KELEMEN ARPAD/AU
E2          2      KELEMEN ARPAD G/AU
E3          16     --> KELEMEN B/AU
E4          1      KELEMEN B I/AU
E5          32     KELEMEN B R/AU
E6          1      KELEMEN B S/AU
E7          2      KELEMEN BARBARA/AU
E8          7      KELEMEN BELA/AU
E9          1      KELEMEN BRAD/AU
E10         2      KELEMEN BRADLEY/AU
E11         1      KELEMEN BRADLEY D/AU
E12         28     KELEMEN BRADLEY R/AU

=> s e1-e12
L6          102    ("KELEMEN ARPAD"/AU OR "KELEMEN ARPAD G"/AU OR "KELEMEN B"/AU
                  OR "KELEMEN B I"/AU OR "KELEMEN B R"/AU OR "KELEMEN B S"/AU OR
                  "KELEMEN BARBARA"/AU OR "KELEMEN BELA"/AU OR "KELEMEN BRAD"/AU
                  OR "KELEMEN BRADLEY"/AU OR "KELEMEN BRADLEY D"/AU OR "KELEMEN
                  BRADLEY R"/AU)

=> d his
(FILE 'HOME' ENTERED AT 12:26:36 ON 18 SEP 2007)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
LIFESCI' ENTERED AT 12:27:04 ON 18 SEP 2007
L1          318    S PROTEORHODOPSIN
L2          86     S "HISTIDINE 75" OR "HIS75"
L3          0      S L1 AND L2
L4          0      S "H75" AND L1
                  E JENSEN R B/AU
L5          150    S E3
                  E KELEMEN B/AU
L6          102    S E1-E12

=> s l1 and l6
L7          10    L1 AND L6

=> dup rem 17
PROCESSING COMPLETED FOR L7
L8          5 DUP REM L7 (5 DUPLICATES REMOVED)

=> d 1-5 ibib ab

L8 ANSWER 1 OF 5 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2005:1350774 HCAPLUS
DOCUMENT NUMBER: 144:77689
TITLE: Photochromic material comprising a
proteorhodopsin apoprotein and retinal analog
Jensen, Rasmus B.; Kelemen, Bradley; Ward,
Donald E., II; Asato, Alfred E.
INVENTOR(S):
PATENT ASSIGNEE(S): Genencor International, Inc., USA; Dow Corning
SOURCE: PCT Int. Appl., 35 pp.
CODEN: PIIXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.           KIND   DATE       APPLICATION NO.        DATE
-----  -----  -----  -----
WO 2005124230        A1    20051229    WO 2005-US20900    20050609
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
```

CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,  
 GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ,  
 LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA,  
 NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK,  
 SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU,  
 ZA, ZM, ZW  
 RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM,  
 AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK,  
 EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT,  
 RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML,  
 MR, NE, SN, TD, TG  
 CA 2569368 A1 20051229 CA 2005-2569368 20050609  
 EP 1753997 A1 20070221 EP 2005-771676 20050609  
 R: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE,  
 IS, IT, LI, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR  
 PRIORITY APPLN. INFO.: US 2004-579180P P 20040610  
 US 2004-622425P P 20041026  
 WO 2005-US20900 W 20050609

OTHER SOURCE(S): MARPAT 144:77689

AB The present invention relates to a photochromic material comprising a proteorhodopsin apoprotein and a retinal analog. In one embodiment, the retinal analog is an azulenic retinoid compound I [R1,R2,R3 = H, C1-4 straight or branched alkyl; n = 1 -4; X1, X2 = H, C1-4 alkyl, F, Cl or CF3; Y = direct bond, p-, m-, or o-phenyl; Z = CHO]. In another embodiment, the retinal analog is other compound that is structurally similarly to all-trans-retinal. The proteorhodopsin apoprotein and the retinal analog form a photochromic material having different spectral properties from those of a corresponding photochromic material formed by the same proteorhodopsin apoprotein and all-trans-retinal. In one embodiment of the application, the retinal analog-containing proteorhodopsin has an absorbance spectrum that does not overlap significantly with that of all-trans-retinal-containing proteorhodopsin. In another embodiment of the application, the retinal analog-containing proteorhodopsin yields a red shifted visual chromophore compared with the all-trans-retinal-containing proteorhodopsin chromophore. The photochromic material of the present invention is useful as an optical data storage carrier, a fraud-proof optical data carrier, security ink, and in other optical applications.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 2 OF 5 HCPLUS COPYRIGHT 2007 ACS on STN  
 ACCESSION NUMBER: 2005:1350735 HCPLUS  
 DOCUMENT NUMBER: 144:97744  
 TITLE: Compositions comprising various  
 proteorhodopsins and/or bacteriorhodopsins and  
 use thereof for photochromic information carrier  
 INVENTOR(S): Bott, Richard R.; Jensen, Rasmus B.; Kelemen,  
 Bradley; Ward, Donald E., II; Whited, Gregory M.  
 PATENT ASSIGNEE(S): Genencor International, Inc., USA; Dow Corning  
 SOURCE: PCT Int. Appl., 36 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005123110	A2	20051229	WO 2005-US20899	20050609
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ,				

LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA,  
 NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK,  
 SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU,  
 ZA, ZM, ZW  
 RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM,  
 AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK,  
 EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT,  
 RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML,  
 MR, NE, SN, TD, TG  
 CA 2569367 A1 20051229 CA 2005-2569367 20050609  
 EP 1753448 A2 20070221 EP 2005-761082 20050609  
 R: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE,  
 IS, IT, LI, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, AL, BA,  
 HR, LV, MK, YU  
 KR 2007031930 A 20070320 KR 2006-725986 20061208  
 PRIORITY APPLN. INFO.: US 2004-579181P P 20040610  
 US 2004-622424P P 20041026  
 WO 2005-US20899 W 20050609

**AB** The present invention provides a solid material comprising an immobilized mixture of two or more proteorhodopsins, two or more bacteriorhodopsins, or one or more bacteriorhodopsin and one or more proteorhodopsins. The proteorhodopsins are selected from the group consisting of all-trans-retinal-containing proteorhodopsins and retinal analog-containing proteorhodopsins; all of which have absorption spectra that do not overlap. The bacteriorhodopsins are selected from the group consisting of all-trans-retinal-containing bacteriorhodopsins and retinal analog-containing bacteriorhodopsins; all of which have absorption spectra that do not overlap. The present invention also provides an optical information carrier, such as an optical data storage material and a fraud-proof optical data carrier, comprising the above-described solid material and a substrate selected from the group consisting of glass, paper, metal, fabric material, and plastic material, wherein said solid material is deposited on said substrate. The present invention further provides security ink comprising one or more hydrophilic polymers and a mixture of various photochromic materials.

L8 ANSWER 3 OF 5 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN  
DUPLICATE 1

ACCESSION NUMBER: 2004-18649 BIOTECHDS

TITLE: New proteorhodopsin mutant having improved optical characteristics, useful as a photochromic all-trans-retinal protein for optical storage devices, or in devices for ATP generation in reactors and desalination of sea water; for use in optical data storage, interferometry and/or photonics

AUTHOR: JENSEN R B; KELEMEN B R

PATENT ASSIGNEE: GENENCOR INT INC; DOW CORNING CORP

PATENT INFO: WO 2004063326 29 Jul 2004

APPLICATION INFO: WO 2003-US38194 26 Nov 2003

PRIORITY INFO: US 2002-429518 26 Nov 2002; US 2002-429518 26 Nov 2002

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2004-544077 [52]

**AB** DERWENT ABSTRACT:

NOVELTY - A proteorhodopsin mutant having improved optical characteristics and comprising a mutation in a conserved residue of a wild-type proteorhodopsin variant, the proteorhodopsin mutant having lower pKr<sub>h</sub> or less difference in maximum absorption wavelength between a basic and an acidic form, in comparison with the wild-type proteorhodopsin variant, is new.

DETAILED DESCRIPTION - A proteorhodopsin mutant may be a proteorhodopsin mutant comprising a sequence of 249, 249, 249, 252, 252, 252, 252, 252, 252, or 252 amino acids (SEQ ID NO: 163,

165, 167, 169, 171, 173, 175, 177, 179, 181 or 183, respectively) given in the specification. INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid sequence encoding the proteorhodopsin mutant above, or comprising a sequence comprising 750, 750, 750, 756, 756, 756, 756, 756, 756, or 756 bp (SEQ ID NO: 164, 166, 168, 170, 172, 174, 176, 178, 180, 182 or 184, respectively) given in the specification; (2) a method for preparing a proteorhodopsin mutant having improved optical characteristics; (3) a method of storing and retrieving optical data; and (4) a light-driven energy generator comprising the proteorhodopsin mutant above, a cell membrane, a source of all-trans-retinal, and a light source, where the proteorhodopsin mutant integrates within the cell membrane to produce an integrated proteorhodopsin mutant, and the integrated proteorhodopsin mutant binds covalently to all-trans-retinal to produce a light absorbing pigment.

**BIOTECHNOLOGY - Preparation (claimed):** Preparing a proteorhodopsin mutant having improved optical characteristics comprises identifying a conserved amino acid residue of a wild-type proteorhodopsin variant, mutagenizing the conserved amino acid residue and obtaining proteorhodopsin mutants, determining the optical characteristics of the proteorhodopsin mutants, and selecting the proteorhodopsin mutant having improved optical characteristics. The conserved residue is a conserved histidine or arginine residue. The conserved amino acid residue is mutagenized by site-directed mutagenesis. The improved optical characteristics are lower pKr<sub>h</sub> or less difference in maximum absorption wavelength between a basic and an acidic form, in comparison with the wild-type proteorhodopsin variant. The wild-type proteorhodopsin variant is a naturally occurring proteorhodopsin variant having a sequence comprising 230-252 amino acids (odd SEQ ID NOS between SEQ ID NO: 1-161) given in the specification, or other proteorhodopsin variants sharing at least 90% amino acid identity to it. The conserved histidine residue is at amino acid position 77 of SEQ ID NO: 1 or at position 75 of a sequence comprising 756 bp (SEQ ID NO: 2) also given in the specification. The proteorhodopsin mutant has a lower pKr<sub>h</sub> in comparison with the wild-type proteorhodopsin variant. The conserved histidine residue is mutated to an amino acid capable of forming a hydrogen bond, where such amino acid is asparagine, glutamine, lysine, arginine, tryptophan, serine, threonine, tyrosine, aspartic acid or glutamic acid, preferably asparagine, glutamine, lysine, tryptophan, aspartic acid, or glutamic acid. The conserved arginine residue is at amino acid position 96 or 94 of SEQ ID NO: 1 or 2, respectively. The proteorhodopsin mutant may have less difference in maximum absorption wavelengths between a basic and an acidic form, in comparison with the proteorhodopsin variant. The conserved arginine residue is mutated to alanine, glutamic acid or glutamine. Preferred Method: Storing and retrieving optical data comprises: (a) providing a film comprising a matrix having the proteorhodopsin mutant above immobilized within; (b) exposing the film to light of a wavelength that is absorbed by the proteorhodopsin mutant at a resting state in a predetermined pattern; (c) converting selective portions of the film to an excited state and storing optical data in it; (d) exposing the film of step (c) to light of a wavelength that is absorbed by the proteorhodopsin mutant at either a resting state or an excited state; and (e) detecting the stored optical data by an optical recording device.

**USE -** The proteorhodopsin mutant can be incorporated into instruments or devices having photochromic applications (e.g., for optical data storage, interferometry and/or photonics), photoelectric applications, and/or phototransport applications. The proteorhodopsin mutant is useful as a photochromic all-trans-retinal protein for optical storage devices, in devices for information storage such as 2-D storage, 3-D storage, holographic storage, or associative storage. The proteorhodopsin mutant is

useful in a device for information processing such as optical bistability/light switching, optical filtering, signal conditioning, neural networks, spatial light modulators, phase conjugation, pattern recognition, interferometry or the like. The proteorhodopsin mutant is useful in devices for ATP generation in reactors, desalination of sea water, and/or conversion of sunlight into electricity. Furthermore, the proteorhodopsin mutant is useful as a replacement for bacteriorhodopsin for a variety of devices/processes that utilize bacteriorhodopsin, e.g., protein-enzyme biochemical optical recording medium and imaging process.

EXAMPLE - No relevant example given. (316 pages)

L8 ANSWER 4 OF 5 HCPLUS COPYRIGHT 2007 ACS on STN  
 ACCESSION NUMBER: 2004:467948 HCPLUS  
 DOCUMENT NUMBER: 141:25187  
 TITLE: Optical information carrier comprising immobilized proteorhodopsin, security ink, and preparation  
 INVENTOR(S): Jensen, Rasmus B.; Kelemen, Bradley R.; McAuliffe, Joseph C.; Smith, Wyatt C.  
 PATENT ASSIGNEE(S): Genencor International, Inc., USA; Dow Corning Corporation  
 SOURCE: PCT Int. Appl., 37 pp.  
 CODEN: PIXXD2  
 DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 2  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004048451	A2	20040610	WO 2003-US38157	20031126
WO 2004048451	A3	20060216		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2507165	A1	20040610	CA 2003-2507165	20031126
AU 2003298777	A1	20040618	AU 2003-298777	20031126
US 2005095605	A1	20050505	US 2003-724264	20031126
EP 1576042	A2	20050921	EP 2003-796535	20031126
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, SK				
JP 2006515683	T	20060601	JP 2004-555825	20031126
PRIORITY APPLN. INFO.:			US 2002-429518P	P 20021126
			WO 2003-US38157	W 20031126

AB The materials comprise hydrophilic polymers and immobilized proteorhodopsin. The material comprises  $\geq 1$  hydrophilic polymers that form a homogeneous phase with proteorhodopsin prior to solidification to a solid form. The hydrophilic polymer may be SiO<sub>2</sub> sol-gel, gelatin, poly(vinyl alc.), agarose, agar, Me cellulose, polyvinyl acetate, polyvinyl pyrrolidone, polyethylene glycol, or a mixture. The solid material having immobilized proteorhodopsin is deposited on a substrate selected from glass, paper, metal, fabric material, plastic material, and used as an optical data storage material or a fraud-proof carrier. A security ink may also comprise proteorhodopsin and  $\geq 1$  hydrophilic polymers.

ACCESSION NUMBER: 2003565238 MEDLINE  
DOCUMENT NUMBER: PubMed ID: 14643930  
TITLE: Proteorhodopsin in living color: diversity of spectral properties within living bacterial cells.  
AUTHOR: Kelemen Bradley R; Du Mai; Jensen Rasmus B  
CORPORATE SOURCE: Genencor International, Inc, 925 Page Mill Road, Palo Alto, CA 94304, USA.  
SOURCE: Biochimica et biophysica acta, (2003 Dec 3) Vol. 1618, No. 1, pp. 25-32.  
Journal code: 0217513. ISSN: 0006-3002.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200401  
ENTRY DATE: Entered STN: 16 Dec 2003  
Last Updated on STN: 21 Jan 2004  
Entered Medline: 20 Jan 2004

AB Proteorhodopsin is a family of over 50 proteins that provide phototrophic capability to marine bacteria by acting as light-powered proton pumps. The potential importance of proteorhodopsin to global ocean ecosystems and the possible applications of proteorhodopsin in optical data storage and optical signal processing have spurred diverse research in this new family of proteins. We show that proteorhodopsin expressed in Escherichia coli is functional and properly inserted in the membrane. At high expression levels, it appears to self-associate. We present a method for determining spectral properties of proteorhodopsin in intact E. coli cells that matches results obtained with detergent-solubilized, purified proteins. Using this method, we observe distinctly different spectra for protonated and deprotonated forms of 21 natural proteorhodopsin proteins in intact E. coli cells. Upon protonation, the wavelength maxima red shifts between 13 and 53 nm. We find that pKa values between 7.1 and 8.5 describe the pH-dependent spectral shift for all of the 21 natural variants of proteorhodopsin. The wavelength maxima of the deprotonated forms of the 21 natural proteorhodopsins cluster in two sequence-related groups: blue proteorhodopsins (B-PR) and green proteorhodopsins (G-PR). The site-directed substitution Leu105Gln in Bac31A8 proteorhodopsin shifts this G-PR's wavelength maximum to a wavelength maximum the same as that of the B-PR Hot75m1 proteorhodopsin. The site-directed substitution Gln107Leu in Hot75m1 proteorhodopsin shifts this B-PR's wavelength maximum to a wavelength maximum as that of Bac31A8 proteorhodopsin.

=> d his

(FILE 'HOME' ENTERED AT 12:26:36 ON 18 SEP 2007)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 12:27:04 ON 18 SEP 2007

L1 318 S PROTEORHODOPSIN  
L2 86 S "HISTIDINE 75" OR "HIS75"  
L3 0 S L1 AND L2  
L4 0 S "H75" AND L1  
E JENSEN R B/AU  
L5 150 S E3  
E KELEMEN B/AU  
L6 102 S E1-E12  
L7 10 S L1 AND L6  
L8 5 DUP REM L7 (5 DUPLICATES REMOVED)

=> s l1 (w)mutant2  
2 IS NOT A RECOGNIZED COMMAND  
The previous command name entered was not recognized by the system.  
For a list of commands available to you in the current file, enter  
"HELP COMMANDS" at an arrow prompt (=>).

=> s l1 (w)mutant?  
L9 6 L1 (W) MUTANT?  
  
=> dup rem 19  
PROCESSING COMPLETED FOR L9  
L10 2 DUP REM L9 (4 DUPLICATES REMOVED)

=> d 1-2 ibib ab

L10 ANSWER 1 OF 2 MEDLINE on STN DUPLICATE 1  
ACCESSION NUMBER: 2007520267 IN-PROCESS  
DOCUMENT NUMBER: PubMed ID: 16808594  
TITLE: Photoisomerization in proteorhodopsin  
mutant D97N.  
AUTHOR: Lenz Martin O; Woerner Andreas C; Glaubitz Clemens;  
Wachtveitl Josef  
CORPORATE SOURCE: Institute for Physical and Theoretical Chemistry, Johann  
Wolfgang Goethe-Universitat Frankfurt, Germany.  
SOURCE: Photochemistry and photobiology, (2007 Mar-Apr) Vol. 83,  
No. 2, pp. 226-31.  
Journal code: 0376425. ISSN: 0031-8655.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
(RESEARCH SUPPORT, NON-U.S. GOV'T)  
LANGUAGE: English  
FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals  
ENTRY DATE: Entered STN: 7 Sep 2007  
Last Updated on STN: 7 Sep 2007

AB The first steps of the photocycle of the D97N mutant of proteorhodopsin (PR) have been investigated by means of ultrafast transient absorption spectroscopy. A comparison with the primary dynamics of native PR and D85N mutant of bacteriorhodopsin is given. Upon photoexcitation of the covalently bound all-trans retinal the excited state decays biexponentially with time constants of 1.4 and 20 ps via a conical intersection, resulting in a 13-cis isomerized retinal. Neither of the two-deactivation channels is significantly preferred. The dynamics is slowed down in comparison with native PR at pH 9 and reaction rates are even lower than for native PR at pH 6, where the primary proton acceptor (Asp97) is protonated. Therefore, the ultrafast isomerization is not only controlled by the charge distribution within the retinal binding pocket. This study shows that in addition to direct electrostatics other effects have to be taken into account to explain the catalytic function of Asp97 in PR on the ultrafast isomerization reaction. This may include sterical interactions and/or bound water molecules within the retinal binding pocket.

L10 ANSWER 2 OF 2 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN  
DUPLICATE 2  
ACCESSION NUMBER: 2004-18649 BIOTECHDS  
TITLE: New proteorhodopsin mutant having  
improved optical characteristics, useful as a photochromic  
all-trans-retinal protein for optical storage devices, or in  
devices for ATP generation in reactors and desalination of  
sea water;  
for use in optical data storage, interferometry and/or  
photonics  
AUTHOR: JENSEN R B; KELEMEN B R  
PATENT ASSIGNEE: GENENCOR INT INC; DOW CORNING CORP

PATENT INFO: WO 2004063326 29 Jul 2004  
APPLICATION INFO: WO 2003-US38194 26 Nov 2003  
PRIORITY INFO: US 2002-429518 26 Nov 2002; US 2002-429518 26 Nov 2002  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2004-544077 [52]

AB DERWENT ABSTRACT:

NOVELTY - A proteorhodopsin mutant having improved optical characteristics and comprising a mutation in a conserved residue of a wild-type proteorhodopsin variant, the proteorhodopsin mutant having lower pKr<sub>h</sub> or less difference in maximum absorption wavelength between a basic and an acidic form, in comparison with the wild-type proteorhodopsin variant, is new.

DETAILED DESCRIPTION - A proteorhodopsin mutant may be a proteorhodopsin mutant comprising a sequence of 249, 249, 249, 252, 252, 252, 252, 252, 252, 252, or 252 amino acids (SEQ ID NO: 163, 165, 167, 169, 171, 173, 175, 177, 179, 181 or 183, respectively) given in the specification. INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid sequence encoding the proteorhodopsin mutant above, or comprising a sequence comprising 750, 750, 750, 756, 756, 756, 756, 756, 756, or 756 bp (SEQ ID NO: 164, 166, 168, 170, 172, 174, 176, 178, 180, 182 or 184, respectively) given in the specification; (2) a method for preparing a proteorhodopsin mutant having improved optical characteristics; (3) a method of storing and retrieving optical data; and (4) a light-driven energy generator comprising the proteorhodopsin mutant above, a cell membrane, a source of all-trans-retinal, and a light source, where the proteorhodopsin mutant integrates within the cell membrane to produce an integrated proteorhodopsin mutant, and the integrated proteorhodopsin mutant binds covalently to all-trans-retinal to produce a light absorbing pigment.

BIOTECHNOLOGY - Preparation (claimed): Preparing a proteorhodopsin mutant having improved optical characteristics comprises identifying a conserved amino acid residue of a wild-type proteorhodopsin variant, mutagenizing the conserved amino acid residue and obtaining proteorhodopsin mutants, determining the optical characteristics of the proteorhodopsin mutants, and selecting the proteorhodopsin mutant having improved optical characteristics. The conserved residue is a conserved histidine or arginine residue. The conserved amino acid residue is mutagenized by site-directed mutagenesis. The improved optical characteristics are lower pKr<sub>h</sub> or less difference in maximum absorption wavelength between a basic and an acidic form, in comparison with the wild-type proteorhodopsin variant. The wild-type proteorhodopsin variant is a naturally occurring proteorhodopsin variant having a sequence comprising 230-252 amino acids (odd SEQ ID NOs between SEQ ID NO: 1-161) given in the specification, or other proteorhodopsin variants sharing at least 90% amino acid identity to it. The conserved histidine residue is at amino acid position 77 of SEQ ID NO: 1 or at position 75 of a sequence comprising 756 bp (SEQ ID NO: 2) also given in the specification. The proteorhodopsin mutant has a lower pKr<sub>h</sub> in comparison with the wild-type proteorhodopsin variant. The conserved histidine residue is mutated to an amino acid capable of forming a hydrogen bond, where such amino acid is asparagine, glutamine, lysine, arginine, tryptophan, serine, threonine, tyrosine, aspartic acid or glutamic acid, preferably asparagine, glutamine, lysine, tryptophan, aspartic acid, or glutamic acid. The conserved arginine residue is at amino acid position 96 or 94 of SEQ ID NO: 1 or 2, respectively. The proteorhodopsin mutant may have less difference in maximum absorption wavelengths between a basic and an acidic form, in comparison with the proteorhodopsin variant. The conserved arginine residue is mutated to alanine, glutamic acid or glutamine. Preferred

Method: Storing and retrieving optical data comprises: (a) providing a film comprising a matrix having the proteorhodopsin mutant above immobilized within; (b) exposing the film to light of a wavelength that is absorbed by the proteorhodopsin mutant at a resting state in a predetermined pattern; (c) converting selective portions of the film to an excited state and storing optical data in it; (d) exposing the file of step (c) to light of a wavelength that is absorbed by the proteorhodopsin mutant at either a resting state or an excited state; and (e) detecting the stored optical data by an optical recording device.

USE - The proteorhodopsin mutant can be incorporated into instruments or devices having photochromic applications (e.g., for optical data storage, interferometry and/or photonics), photoelectric applications, and/or phototransport applications. The proteorhodopsin mutant is useful as a photochromic all-trans-retinal protein for optical storage devices, in devices for information storage such as 2-D storage, 3-D storage, holographic storage, or associative storage. The proteorhodopsin mutant is useful in a device for information processing such as optical bistability/light switching, optical filtering, signal conditioning, neural networks, spatial light modulators, phase conjugation, pattern recognition, interferometry or the like. The proteorhodopsin mutant is useful in devices for ATP generation in reactors, desalination of sea water, and/or conversion of sunlight into electricity. Furthermore, the proteorhodopsin mutant is useful as a replacement for bacteriorhodopsin for a variety of devices/processes that utilize bacteriorhodopsin, e.g., protein-enzyme biochemical optical recording medium and imaging process.

EXAMPLE - No relevant example given. (316 pages)

=> d his

(FILE 'HOME' ENTERED AT 12:26:36 ON 18 SEP 2007)

FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 12:27:04 ON 18 SEP 2007

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L4       0 S "H75" AND L1  
          E JENSEN R B/AU  
L5       150 S E3  
          E KELEMEN B/AU  
L6       102 S E1-E12  
L7       10 S L1 AND L6  
L8       5 DUP REM L7 (5 DUPLICATES REMOVED)  
L9       6 S L1 (W)MUTANT?  
L10      2 DUP REM L9 (4 DUPLICATES REMOVED)

	Document ID	Kind Codes	Source	Issue Date	Page s	Title
1	US 2005009560 5 A1		US- PGPUB	20050505	241	Proteorhodopsin mutants with improved optical characteristics
2	US 2004022332 3 A1		US- PGPUB	20041111	20	Optical information carrier comprising immobilized proteorhodopsin

	Document ID	Kind Codes	Source	Issue Date	Page s	Title
1	US 2006024042 3 A1		US- PGPUB	20061026	79	Isolation and cloning of dna from uncultivated organisms
2	US 2005025012 8 A1		US- PGPUB	20051110	26	Membranes incorporating recognition moieties
3	US 2005014876 2 A1		US- PGPUB	20050707	13	Rapid and inexpensive method for the purification of proteorhodopsin
4	US 2005009560 5 A1		US- PGPUB	20050505	241	Proteorhodopsin mutants with improved optical characteristics
5	US 2004022332 3 A1		US- PGPUB	20041111	20	Optical information carrier comprising immobilized proteorhodopsin
6	US 2003010437 5 A1		US- PGPUB	20030605	349	Light-driven energy generation using proteorhodopsin
7	US 7253268 B2		USPAT	20070807	215	Light-driven energy generation using proteorhodopsin

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1	US 2005009560 5 A1		US- PGPUB	20050505	241	Proteorhodopsin mutants with improved optical characteristics
2	US 2004022332 3 A1		US- PGPUB	20041111	20	Optical information carrier comprising immobilized proteorhodopsin

	L #	Hits	Search Text
1	L1	168	"his75" or "h75" or "histidine 75"
2	L2	7	proteorhodopsin\$2
3	L3	1	l1 same l2
4	L4	2	l2 adj (mutant? or variant?)
5	L5	3544 7	JENSEN KELEMEN
6	L6	2	l2 and l5